THE EFFECTS OF LITHIUM CHLORIDE ON THE FERTILIZED EGGS OF NEREIS LIMBATA

CATHERINE HENLEY

Marine Biological Laboratory, Woods Hole, Mass., and Department of Biology, The Johns Hopkins University

Introduction

The action of the lithium ion on the eggs of certain animals has long been known. Using a number of lithium salts, Herbst (1892) obtained abnormal embryos of two types. In the first group, the endoderm was found not inside the body, but outside; these forms are designated "exogastrulae." Larvae of the second group showed an apparent conversion of ectoderm into endoderm so that in extreme cases, the whole blastula wall was endodermized (Herbst, 1892, 1893, 1943). Herbst concluded that this was a specific and typical affect of lithium, a view shared by Gurwitsch (1895), Morgan (1902), and other early investigators.

Studying the effects of 0.2 per cent to 1.0 per cent solutions of lithium chloride on frog and toad eggs, Gurwitsch found that in the resulting embryos, gastrulation was abnormal, but no exogastrulae were produced. Morgan (1902), using similar concentrations of lithium, obtained embryos in which the black hemisphere had sunk into the interior of the egg, but here again there was no definite indication of exogastrulation. Experiments by Holtfreter (1931) showed that amphibian exogastrulae could be obtained if the blastulae were treated with a modified Ringer solution. Further experiments to ascertain the effects of lithium on the amphibian egg were carried out by Bellamy (1919) who observed a number of cases of fusion of the lateral sense organs of the larvae, as well as abnormalities in gastrulation. Töndury (1938) treated urodele embryos in early stages of gastrulation with LiCl and described a high percentage of head and foregut abnormalities in the larvae. Still another effect of lithium on urodele eggs was observed by Cohen (1938) who treated early gastrulae or late blastulae and observed cases in which the myotomes formed a continuous sheet across the midline, separating the nerve cord and notochord. He also obtained embryos in which exogastrulation had occurred, seemingly as a result of the treatment with lithium.

The effects of LiCl on the eggs of the pond snail, *Limnaea stagnalis*, were studied by Raven (1942) who was able to produce forms which he designates as exogastrulae. He also describes a number of larvae in which varying abnormalities of the eyes were apparent. These experiments are of interest in connection with the present series because the egg of Limnaea, like that of Nereis, is an example of the so-called "mosaic" type of development.

A wide variety of chemical and physical agents has since been found to produce echinoderm exogastrulae, thus invalidating the theory of an ion specificity. These agents include hypotonic sea water, isotonic solutions of magnesium chloride and lithium chloride, and combinations of isotonic solutions of magnesium chloride, so-

dium chloride, potassium chloride, and calcium chloride as studied by Waterman (1932) ; solutions of nickel chloride and copper chloride (Waterman, 1937) ; lithium in low concentrations augmented by carbon monoxide (Runnström, 1935) ; and 97 per cent carbon monoxide with 3 per cent oxygen in the presence of light (Runnström, 1928a). MacArthur (1924) treated sea urchin eggs with calcium chloride, stale or diluted sea water, copper sulfate, mercuric chloride and potassium cyanide, and obtained exogastrulae. Using "auxin," glycogen and potassium chlorate, Motomura (1934) likewise obtained echinoderm exogastrulae.

Most of the eggs previously tested with lithium have been those of echinoderms and amphibians, both of which are characterized by an invaginative type of gastrulation. It seemed, therefore, that an egg showing a strictly epibolic form of gastrulation should be tested, in an effort to ascertain if the same effect could be produced. The following experiments were performed to study the effects of lithium on the fertilized eggs of the annelid, *Nereis limbata*.[1]

Methods

Gametes of the heteronereis form of *Nereis limbata* were obtained at Woods Hole from June to September, during appropriate phases of the moon. Usually the eggs from one female were sufficient for an average experimental series; they were inseminated according to the methods outlined by Just (1939) and were then washed with freshly drawn, filtered sea water.

The fertilized eggs were allowed to remain undisturbed for a period of 75 minutes after insemination; at the end of this time (shortly before the first cleavage), they were transferred with as little sea water as possible to a series of 25 cc. stender dishes, to which the various solutions of LiCl were then added. Appropriate controls were kept in all series, these being cultured in filtered aerated sea water which was changed at daily intervals. A stock solution of 0.54 M LiCl in distilled water was used for all experimental mixtures; such a solution is approximately isotonic in all dilutions with sea water. Experimental mixtures were designated according to the percentage of this stock solution used—e.g., a "15 per cent solution" indicates that 85 cc. of filtered aerated sea water were added to 15 cc. of the 0.54 M LiCl stock solution. About 15 cc. of the mixture were added to each stender dish; at intervals ranging from 15 minutes to 36 hours, the solutions were decanted carefully and the eggs washed in three or four changes of sea water. LiCl-sea water mixtures ranging from 2 per cent to 100 per cent were tested for varying periods of time. No attempt was made to control the room temperature, which varied from 20° C. to 28° C. However, all cultures were kept in moist chambers which stood in running sea water; the temperature of this sea water averaged about 20° C., and did not vary more than one or two degrees at any time during the entire series of experiments.

Less extensive test series were also conducted for purposes of comparison, using the eggs of Nereis from which the vitelline membrane had been removed, according to the method described by Costello (1945a).

[1] The author wishes to express sincere appreciation to Prof. D. P. Costello for advice and encouragement throughout the course of the investigation, to Prof. Viktor Hamburger and Dr. Charles Packard for use of laboratory space at the Marine Biological Laboratory during the summers of 1944 and 1945, to Prof. B. H. Willier for his kindly interest, and to Mr. John S. Spurbeck for valuable assistance with the drawings.

Results

The most striking characteristic of the experimental larvae is the complete absence of exogastrulation, or of any definite evidence of true vegetalization. Abnormal larvae occurred to some extent in almost all the cultures; these abnormalities included the absence of an apical tuft, lengthening of the prototrochal cilia (Fig. 2A), absence of these cilia in varying degrees (Fig. 2), absence or abnormality of the eye spots (Fig. 3), deficiencies in the number of prototrochal cells (Fig. 3), atypical seta sacs with derangements in the number and position of these organs in older larvae (Fig. 4), and abnormalities in the number and position of the oil droplets (Figs. 2C, 3D, 4B). Most of the experimental larvae had abnormalities of the anal and prototrochal pigments; these abnormalities included both the absence of pigment and an abnormal concentration of pigment granules in various regions of the larvae (Figs. 2, 3, 4, 5). The specific occurrence of these anomalies was roughly proportional to the severity of treatment, being far more marked in cultures which

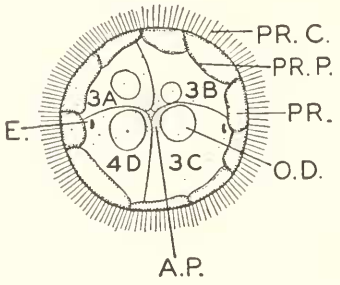

Figure 1. A normal Nereis trochophore at about 30 hours; polar view. Pr. C. = Prototrochal cilia; Pr. P. = Prototrochal pigment; Pr. = Prototrochal cell; O. D. = Oil droplet; A. P. = Anal pigment; E. = Eye spot; 3A, 3B, 3C, 4D = Entomeres. This and all subsequent text-figures are semi-diagrammatic, based on camera lucida drawings of the living larvae.

were exposed to higher concentrations for sublethal periods. No evidence of twinning was observed.

Complete data describing the results of treatment with the varying concentrations for varying periods are contained in Table I. It is evident that concentrations of 2 per cent to 4 per cent acting for periods up to 12 hours produce larvae which are almost completely normal; similar trochophores result when 5 per cent to 10 per cent solutions are applied for one to 5 hours. However, when allowed to act for periods of 5 to 24 hours, the same concentrations affect the eggs so that the resulting larvae show marked deficiencies in the number of prototrochal cells, and are usually completely devoid of the normal anal and prototrochal pigment. Abnormalities such as these occur quite commonly in all series; some extreme examples are shown in Figure 2. For purposes of comparison, a normal trochophore is shown in Figure 1. Accurate quantitative observations of the exact deficiency in the number of prototrochal cells were impossible in most cases.

Twelve per cent solutions acting for periods of 1½ to 5 hours result in trochophores which are essentially normal, but if allowed to remain on the eggs for 5 to 24 hours, bring about pigment and prototrochal anomalies of the type described above.

TABLE I

Effects of lithium treatment of Nereis eggs

Percentage[2]	Duration of treatment in hours	Appearance of 26–30 hour larvae
2%	1½	Normal
	5	Normal
	8	Mostly normal; some with abnormalities in number and position of oil droplets
	23	Mostly normal; some with abnormalities in number and position of oil droplets
3	8	Many with incomplete prototroch and with abnormalities in number and position of oil droplets
	10	Many with incomplete prototroch and with abnormalities in number and position of oil droplets
	24	Abnormal distribution of oil droplets, otherwise normal
4	1½	Normal
	5	Mostly normal
	8	Mostly normal
	12	Normal in form; somewhat sluggish in movements
5	14	Normal
	16	Mostly normal
	18	Mostly normal
	20	Some prototrochal and pigment abnormalities
	22	Pigment and prototrochal abnormalities
	26	Considerable variation in amount of pigment present
	28	Variations in amount of pigment present
	30	More marked pigment abnormalities than after 28-hour treatment
	32	Pigment and prototrochal abnormalities
	36	Dead
6	1½	Normal
	5	Normal, but sluggish in movements
	8	Mostly normal
	12	Normal, but sluggish in movements
	23	Quite abnormal; prototrochal and pigment abnormalities
7	1	Normal
	2	A very few cases with oil droplet deficiencies
	5	Some cases with oil droplet deficiencies
	24	Prototrochal cell deficiencies; sluggish in movements; pigment often absent
8	1½	Normal
	5	Some cases with prototrochal and pigment abnormalities
	8	More marked pigment abnormalities
	12	Severe prototrochal deficiencies; no pigment
	23	Severe prototrochal deficiencies; no pigment. Oil droplets abnormal in number and position. Many larvae without apical tuft

[2] Percentages in the table refer to percentages of 0.54 M stock solution of lithium chloride with sea water: 2 per cent = 2 cc. 0.54 M LiCl + 98 cc. sea water.

TABLE I—*Continued*

Percentage[2]	Duration of treatment in hours	Appearance of 26–30 hour larvae
10	1	Some localized deposits of pigment; some pigment present in most cases
	4	Some pigmented, some unpigmented. A few cases of abnormality in amount and position of anal pigment
	8	Pigment abnormalities; prototrochal cells seem fairly complete
	12	Pigment abnormalities; prototroch fairly normal
	14	No pigment; marked defects in number of prototrochal cells
	16	No pigment; marked defects in number of prototrochal cells
	18	No pigment, no cilia, no apical tuft
	20	No pigment, no apical tuft. Abnormalities in number and position of oil droplets
	24	No pigment, no cilia, no apical tuft. Abnormalities in number and position of oil droplets
	26	No pigment; marked prototrochal deficiencies. No apical tuft; oil droplets very abnormal in number and position
	28	No pigment; prototrochal defects marked. Oil droplets abnormal in number and position
	36	Dead
12	1½	Fairly normal; some abnormalities in number of prototrochal cells and oil droplets
	5	Many deficiencies of pigment and prototroch; oil droplets abnormal in number and position
	8	Pigment deficiencies; prototroch and oil droplets abnormal in number and position
	12	Some abnormalities in distribution of oil droplets; no pigment. Prototrochal deficiencies; no apical tuft
	23	Dead
14	1½	Mostly normal; some pigment deficiencies
	5	No pigment; marked prototrochal deficiencies
	8	No pigment; prototrochal deficiencies; no apical tuft
	23	Dead
15	1	Mostly unpigmented; prototrochal deficiencies. Anal pigment present in many cases. Oil droplets abnormal in number and position
	2	Unpigmented; prototrochal deficiencies; anal pigment present in some cases. Oil droplet abnormalities
	4	More marked pigment defects; severe prototrochal deficiencies. Some few cases of endodermal extrusion
	6	Highest incidence of endodermal extrusion: 2%–7%
	8	Somewhat fewer cases of extrusion than in 6-hour culture
	10	Some few cases of endoderm extrusion
	12	A very few cases of endodermal extrusion
	14	No pigment; marked prototrochal defects. No apical tuft.
	16	No pigment; prototrochal defects; no apical tuft
	20	No cilia, no pigment, no apical tuft. Severe prototrochal deficiencies
	24	No pigment, no cilia, no apical tuft

TABLE I—*Continued*

Percentage‡	Duration of treatment in hours	Appearance of 26–30 hour larvae
17	1	Anal pigment present; prototrochal pigment absent in many cases. Considerable number of oil droplet abnormalities
	2	No pigment; oil droplet abnormalities; prototrochal deficiencies; no apical tuft
	3	No pigment; oil droplet abnormalities in number and position. Prototrochal deficiencies, no apical tuft
	6	Marked oil droplet deficiencies and abnormalities in position; no pigment. No apical tuft; prototrochal cell deficiencies
	8	Many amorphous forms; no cilia; abnormalities in number and position of oil droplets
	10	No cilia, no pigment, no apical tuft
	14	Dead
20	1	Normal
	2	Pigment abnormalities; some localization of pigment
	4	No pigment; prototrochal cell deficiencies; apical tuft apparently missing; oil droplets abnormal in number and position
	8	Marked oil droplet deficiencies; severe prototrochal defects
	12	Oil droplet abnormalities; marked prototrochal deficiencies
	14	Dead
25	1	Prototrochal cell deficiencies; some pigment defects
	2	No pigment; prototrochal cell deficiencies; abnormalities in number and position of oil droplets
	4	Severe prototrochal deficiencies; no apical tuft. No anal pigment
	8	Prototrochal deficiencies; pigment abnormal. Oil droplet abnormalities in number and position
	12	Marked prototrochal cell deficiencies, no pigment, no apical tuft
	14	Dead
30	2	No pigment; marked prototrochal deficiencies. No apical tuft in most cases
	4	Dead
35	2	No pigment; marked prototrochal deficiencies. No apical tuft
40–90	1	Dead
50	½	No pigment; prototrochal deficiencies. Apical tuft usually absent
100	¼	Prototrochal and pigment deficiencies
	½	Dead

Treatment of the eggs with 15 per cent solutions for one to 4 hours produces pigment and prototrochal abnormalities, but after 6, 8, and 10 hours of treatment, the resulting larvae are marked by peculiar endodermal derangements which were at first thought to be the result of exogastrulation. However, closer examination of these cases revealed that the extruded cytoplasm was non-cellular insofar as could be determined, although it had the characteristic "glassy" appearance of endodermal cytoplasm; usually these larvae were marked by the complete absence of cilia (Fig. 5).

Frequently cases were observed in which the endoderm extended up under the surmounting cap of ectoderm. The cleavage pattern of the experimental eggs of this series showed no abnormality through the fourth cleavage. Repeated experiments with this treatment and with exposure of the eggs to concentrations of 12 per cent and 17 per cent for varying periods of time definitely established the fact that the abnormalities occur only after the treatment described above. It does not seem likely that such an extremely narrow range of dosage would be necessary for the production of true exogastrulae, inasmuch as these forms are produced in sea urchin eggs after a rather wide variety of treatments.

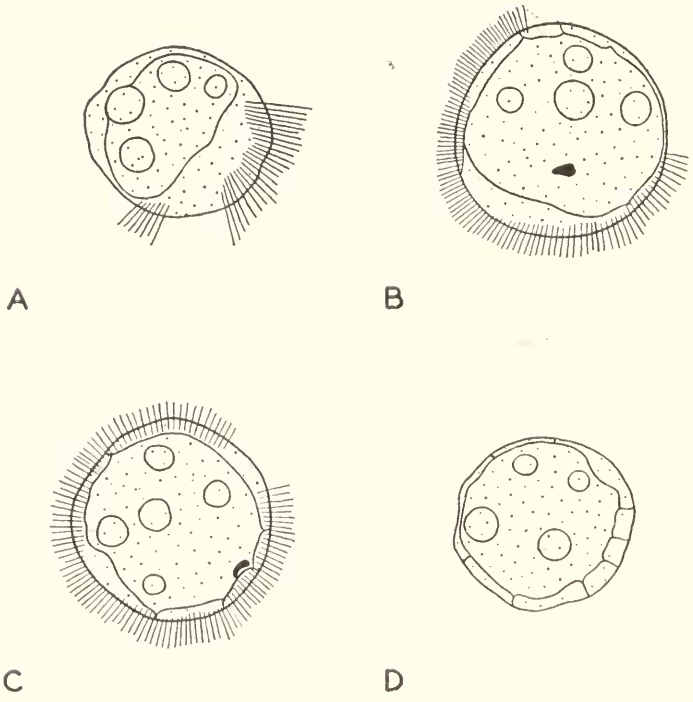

FIGURE 2. Typical 26–30 hour abnormal trochophores, showing anomalies in ciliary band, pigmentation and eye spots. Anal pigment and prototrochal pigment are absent in all cases; Figure 2A shows lengthening of the prototrochal cilia.

Exposure of the eggs to a 17 per cent solution resulted in generally abnormal trochophores after a treatment of one hour, and in more pronounced pigment and prototrochal abnormalities if treated for longer periods, up to the lethal point at 14 hours. No larvae with the endodermal derangements described above were noted in any of the cultures in this series. The effects of a 20 per cent solution for comparable periods of time approximate those described for a 17 per cent treatment; a concentration of 25 per cent produces pigment and prototrochal abnormalities after treatment for 1, 2, 4 and 8 hours. These defects also result from treatment for 2 hours with a 30 per cent solution. Concentrations of 40 per cent to 90 per cent

are lethal after treatment for one hour, and a 100 per cent solution has the same effect after ½ hour; the 100 per cent concentration produces pigment and prototrochal defects if applied for 15 minutes, so that its range of dosage is apparently very narrow. The reduction of the prototroch seems to increase quantitatively with an increase in the concentration of lithium and the duration of application.

Denuded Nereis eggs proved to be extremely sensitive to the effects of lithium, even in low concentrations for short periods of time. In all series, the controls showed considerable abnormality in shape and in the distribution of pigment, so that

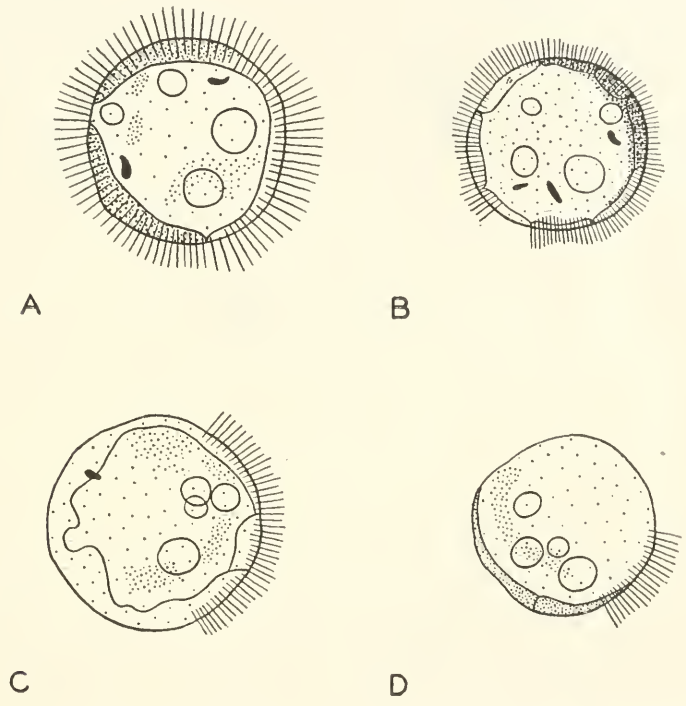

FIGURE 3. Abnormalities involving pigment, eye spots, cilia, and oil droplets after various treatments with lithium. Figure 3A is almost normal except for the heavy concentrations of anal and prototrochal pigment; Figure 3B indicates an abnormality in the number and position of the eye spots and in the size of these structures.

it is difficult to come to any definite decision as to the specific effects brought about by the lithium. However, in the cases in which membrane removal was not complete, the larvae survived fairly well, and in none of these cases were any indications of exogastrulation observed. After treatment for 2 hours with 15 per cent LiCl, some of the completely denuded larvae seemed to show signs of exogastrulation, although no conclusions can be drawn from this because of the small number of cases. Treatment of the denuded eggs with 5 per cent solutions for periods longer than 4 hours killed the eggs in late stages of cleavage, and no definite effects could be noted in larvae surviving in cultures which had been exposed for periods of 1 and 2 hours.

Discussion

From the foregoing results, it is apparent that the main effect of the lithium is to produce a reduction in the number of prototrochal cells, and in the quantity of anal and prototrochal pigments, together with effects on the eye spots and cilia. The absence of the apical tuft in most cases of prolonged or concentrated treatment indicates that the 1a-1d cells may be affected directly or indirectly, since it is known from the observations of Wilson (1892) that this quartet is associated with the formation

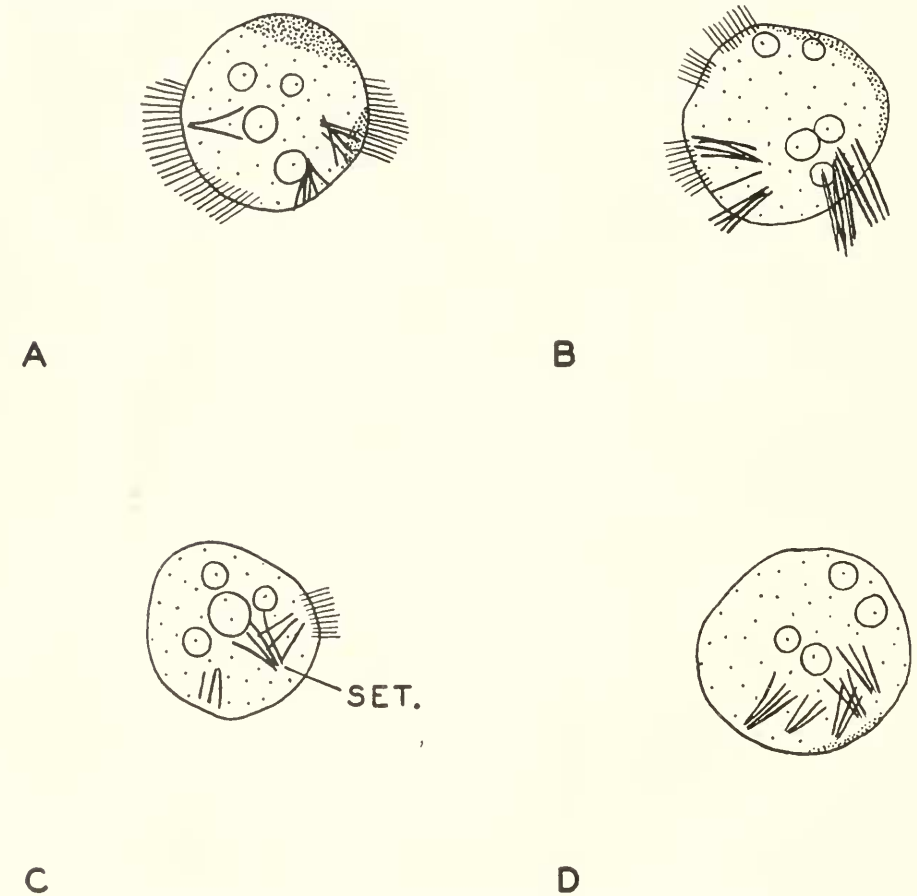

Figure 4. Marked abnormalities in setae of older larvae (ca. 53 hours). Figure 4B also shows an atypical number of oil droplets. Set. = Setae.

of the apical tuft. It is possible that the abnormalities observed in the seta sacs of older larvae (Fig. 4) may be due to the early action of the ion on the material destined to be incorporated into the 2d cell.

The absence of any true cases of exogastrulation in these experiments is quite striking. As was noted above, most of the forms in which this abnormality has been observed are marked by a type of gastrulation in which invagination plays a main

role. In the amphibian, formation of the endoderm is accomplished by a complex series of cell movements, as a result of which a directed and organized migration of cells brings about invagination of most of the vegetal hemisphere at the region of the blastopore. Meanwhile, the animal cells grow down to cover the vegetal region, thus forming the ectoderm of the embryo. The yolk material itself is invaginated in these forms and comes to be enclosed by the endoderm. Gastrulation in the echinoderms is likewise thought by Herbst (1893) to be brought about by an invaginative process. The strictly epibolic form of gastrulation exhibited by the egg of Nereis, on the other hand, is accomplished by a downgrowth of the ectomeres, so that even-

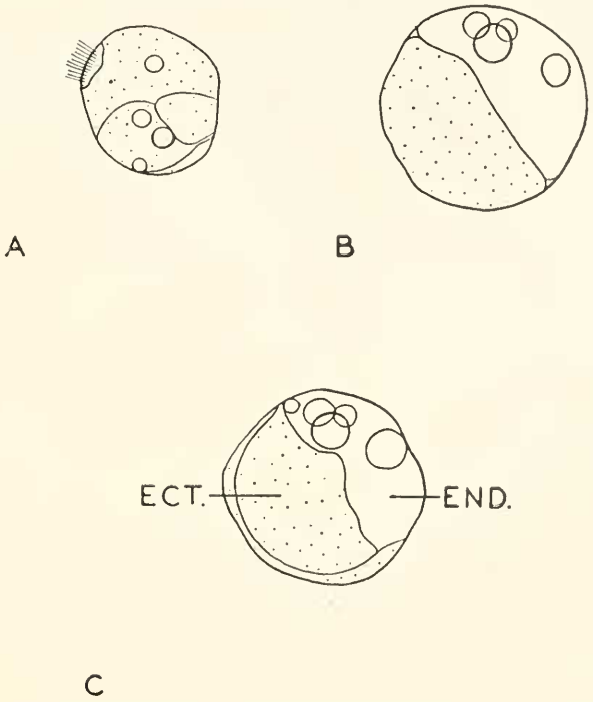

FIGURE 5. Abnormal trochophores resulting from treatment of eggs with 15 per cent LiCl for 6, 8 and 10 hours. Figure 5A shows no particular endodermal derangements, but has only one small tuft of cilia and is quite atypical in shape. Figures 5B and 5C show examples of "endodermal extrusions." Cell boundaries were not visible except as indicated. Ect. = Ectoderm; End. = Endoderm.

tually they cover the four entomeres. Such a movement seems to be associated with the presence of a "cellular affinity" as postulated by Costello (1940, 1945a) in connection with his experiments on the development of isolated blastomeres of this egg. Since no exogastrulation occurred, it may be assumed that this dynamic association of ectomeres and entomeres is not radically disturbed. However, the abnormalities in the number and position of the oil droplets in the four entomeres indicate that the lithium may exert some effect on the process of cytoplasmic segregation (Costello, 1945b) preceding the formation of the 3A, 3B, 3C and 4D cells.

No distinct line of demarcation can be drawn between the direct and the indirect effects of lithium in these experiments, since the ion is brought into contact with the egg before cleavage has occurred, and, in the case of the longer exposures, may remain until considerably after the completion of gastrulation. Runnström (1928b), observing lithium-treated sea urchin eggs under dark-field illumination, presented evidence that the element actually penetrates the cells. Spek (1918) maintained that the action of the lithium ion was brought about through its production of a precipitation and swelling effect on the surface of the vegetative cells. Thus, the exact mode of action remains obscure, but it appears fairly clear in the case of the egg of Nereis that exogastrulation is not produced, at least not in cases where the vitelline membrane is present.

The effects produced on the pigment of the trochophores seem to be at random, since the anal pigment may occur without the presence of the prototrochal pigment, and vice versa—or both may be present in varying degrees. This action possibly is related to the orientation of the eggs with respect to the bottom of the dish, or to each other; although no particular effort was made to keep the eggs suspended in the solution, the jelly serves to support them during the early stages of development, thus allowing relatively free access of the lithium to all surfaces.

Summary

1. The fertilized eggs of *Nereis limbata* were treated with mixtures of sea water and a 0.54 M stock solution of LiCl, ranging from 2 per cent to 100 per cent for periods of 15 minutes to 36 hours. Treatment was begun 75 minutes after insemination of the eggs, shortly before the appearance of the first cleavage.

2. No exogastrulae were observed. There were a few cases of marked abnormalities in the endodermal components of the trochophores within a very narrow range of treatment (15 per cent for 6, 8 and 10 hours).

3. The main abnormalities observed in the experimental larvae were: Absence of the apical tuft, lengthening of the prototrochal cilia, absence of these cilia in varying degrees, abnormalities in the anal and prototrochal pigments, absence or abnormality of the eye spots, deficiencies in the number of prototrochal cells, atypical seta sacs, abnormalities in the number and position of oil droplets. The degree of abnormality in these cases seemed to be roughly proportional to the severity of treatment.

LITERATURE CITED

Bellamy, A. W., 1919. Differential susceptibility as a basis for modification and control of early development in the frog. *Biol. Bull.*, **37**: 312–361.

Cohen, A., 1938. Myotome fusion in the embryo of Amblystoma punctatum after treatment with lithium and other agents. *Jour. Exp. Zool.*, **79**: 461–472.

Costello, D. P., 1940. Gastrulation of isolated blastomeres of Nereis eggs. *Anat. Rec.* (Suppl.), **78**: 132–133.

Costello, D. P., 1945a. Experimental studies of germinal localization in Nereis. I. The development of isolated blastomeres. *Jour. Exp. Zool.*, **100**: 19–66.

Costello, D. P., 1945b. Segregation of oöplasmic constituents. *Jour. Elisha Mitchell Scientific Soc.*, **61**: 277–289.

Gurwitsch, A., 1895. Ueber die Einwirkung des Lithionchlorids auf die Entwicklung der Frosch- und Krötencier (R. fusca und Bufo vulg.). *Anat. Anz.*, **11B**: 65–70.

Herbst, C., 1892. Experimentelle Untersuchung über den Einfluss der veränderten chemischen Zusammensetzung des umgebenden Mediums auf die Entwicklung der Thiere. 1 Theil. Versuche an Seeigeleiern. *Zeit. Wiss. Zool.*, **55**: 446–518.

HERBST, C., 1893. Experimentelle Untersuchung über den Einfluss der veränderten chemischen Zusammensetzung des umgebenden Mediums auf die Entwicklung der Thiere. 2 Theil. Weiteres über die morphologische Wirkung der Lithiumsalze und ihre theoretische Bedeutung. *Mitt. Zool. Stat. Naples*, **11**: 136–220.

HERBST, C., 1943. Die Bedeutung der Salzversuche für die Frage nach der Wirkungsart der Gene. Zusammenschau einer Hydrationsbzw. Mikrolaboratorientheorie der Genwirkung. *Arch. f. Entw. der Org.*, **142**: 319–378.

HOLTFRETER, J. 1931. Über die Aufzucht isolierter Teile des Amphibienkeimes. II. Züchtung von Keimen und Keimteilen in Salzlösung. *Arch. f. Entw. der Org.*, **124**: 404–466.

JUST, E. E., 1939. *Basic methods for experiments on egg of marine animals.* Philadelphia: P. Blakiston's Sons and Co., Inc. 89 pp.

MACARTHUR, J. W., 1924. An experimental study and a physiological interpretation of exogastrulation and related modifications in echinoderm embryos. *Biol. Bull.*, **46**: 60–87.

MORGAN, T. H., 1902. The relation between normal and abnormal development of the embryo of the frog, as determined by the effect of lithium chloride in solution. *Arch. f. Entw. der Org.*, **16**: 691–712.

MOTOMURA, I., 1934. Über die Exogastrulabildung beim Seeigelkeim durch Auxin, Glycogen, und $KClO_3$. *Sci. Rep. Tôhoku Imp. Univ. Series 4, Biology*, **9**: 123–130.

RAVEN, C. P., 1942. The influence of lithium upon the development of the pond snail, Limnaea stagnalis L. *Proc. Nederl. Akad. van Wetens.*, **45**: 856–860.

RUNNSTRÖM, J., 1928a. Zur experimentellen Analyse der Wirkung des Lithiums auf den Seeigelkeim. *Acta Zool.*, **9** (3): 365–424.

RUNNSTRÖM, J., 1928b. Plasmabau und Determination bei dem Ei von Paracentrotus lividus Lk. *Arch. f. Entw. der Org.*, **113**: 556–581.

RUNNSTRÖM, J., 1935. An analysis of the action of lithium on sea urchin development. *Biol. Bull.*, **68**: 378–384.

SPEK, J., 1918. Differenzen im Quellungszustand der Plasmakolloide als eine Ursache der Gastrulainvagination, sowie der Einstülpungen und Faltungen von Zellplatten überhaupt. *Kolloidchem. Beih.*, **9**: 259–399.

TÖNDURY, G., 1938. Weitere Beiträge zur Frage der Kopfentwicklung bei Urodelen. II. Erzeugung von Mikrokephalie durch Einwirkung von Lithiumchlorid auf die Gastrula von Triton alpestris. *Arch. f. Entw. der Org.*, **137**: 510–539.

WATERMAN, A. J., 1932. Effects of isotonic salt solutions upon the development of the blastula of the sea urchin, Paracentrotus lividus. *Arch. de Biol.*, **43**: 471–519.

WATERMAN, A. J., 1937. Effects of salts of heavy metals on development of the sea urchin, Arbacia punctulata. *Biol. Bull.*, **73**: 401–420.

WILSON, E. B., 1892. The cell lineage of Nereis. A contribution to the cytogeny of the annelid body. *Jour. Morph.*, **6**: 361–480.